APTO-253 is a new addition to the repertoire of drugs that can exploit DNA BRCA1/2 deficiency

Poster: #4840

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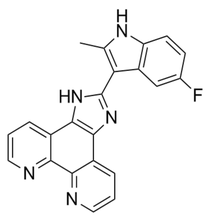
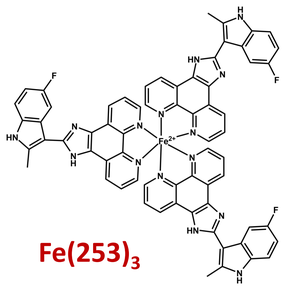
INTRODUCTION

- **APTO-253 is a small molecule with anti-proliferative activity against malignant cell lines derived from a wide range of human malignancies.**
- **APTO-253 was advanced into a Phase 1 clinical trial in patients with advanced solid tumors (NCT123226). It was well tolerated and produced evidence of antitumor activity in patients but did not produce myelosuppression even at the maximum tested dose.**
- **APTO-253 was also advanced into a Phase 1 clinical trial in patients with relapsed/refractory hematologic malignancies (NCT02267863) with emphasis on acute myeloid leukemia.**
- **APTO-253 was found to convert intracellularly to a complex containing one molecule of iron and three molecules of APTO-253 [Fe(253)$_3$].**
- **The purpose of this project was to determine the mechanisms of action and basis for resistance to APTO-253 so as to identify synthetic lethal interactions that can guide combination studies.**



APTO-253



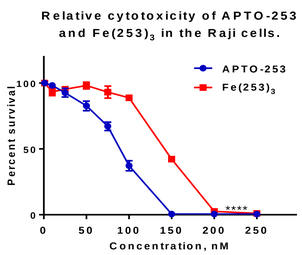
Fe(253)$_3$

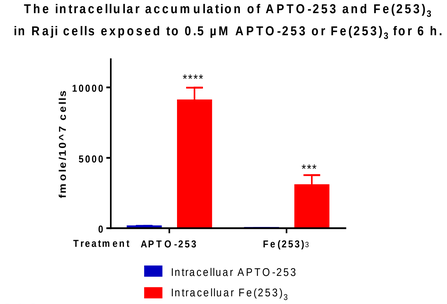
MATERIALS and METHODS

- **Cytotoxicity study** : Cells were plated and treated with the indicated drugs in 96 well plates for 5 days. Cell viability was measured using CellTiter 96® AQ$_{ueous}$ one solution cell proliferation assay purchased from Promega, and IC$_{50}$ values were calculated using GraphPad Prism 6 software.
- **RNA-seq**: Total cellular RNA was isolated using the RNeasy mini kit (QIAGEN, Valencia, CA) from 3 independent samples for each experiment. For RNA-seq samples were submitted to the UCSD IGM Genomics Center (http://igm.ucsd.edu/genomics/) for library generation and validation using Agilent Bioanalyzer. Sequencing was performed on Illumina Sequencer HiSeq4000. Bioinformatic analysis was conducted by Oregon Health & Science University. RNA Sequencing data was deposited to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111928
- **Cellular Pharmacology of APTO-253:** Cells exposed to APTO-253 or Fe(253)$_3$ were homogenized in acetonitrile containing 5 ng of deuterated APTO-253 standard. Samples were analyzed at the UCSD Molecular Mass Spectrometry Facility employing an Agilent 1260 liquid chromatograph system coupled with a Thermo LCQdeca mass spectrometer using positive ion mode electrospray ionization as the ion source.

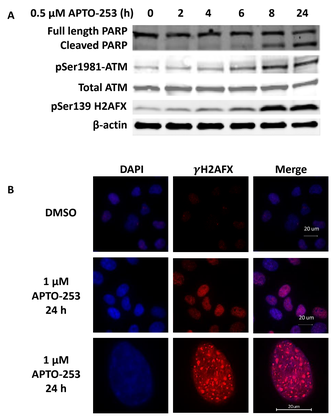
RESULTS

Fe(253)$_3$ is an active intracellular form of APTO-253





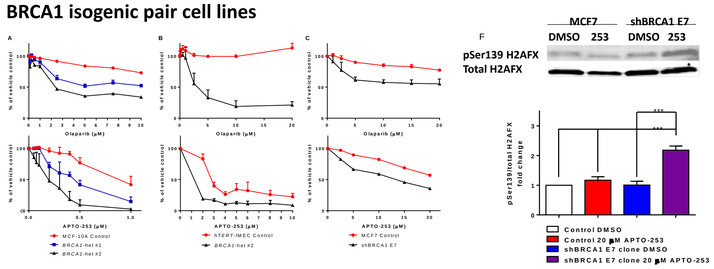
APTO-253 causes DNA damage



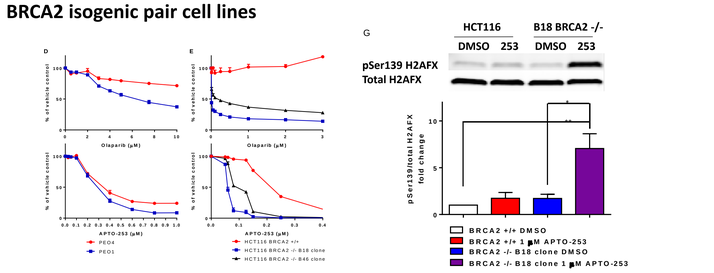
(A) The accumulation of phospho-ATM, γ-H2AFX and cleaved PARP in the Raji cells exposed to APTO-253. (B) Representative immunofluorescent images of nuclear foci formation comparing DMSO- and APTO-253-treated CAOV3 cells. (C) γ-H2AFX foci numbers per cell; N = 100. (D) Percent tail DNA in the Raji cells treated with DMSO or 0.5 μM APTO-253, N = number of cells examined.

BRCA1/2 deficient cells are hypersensitive to APTO-253

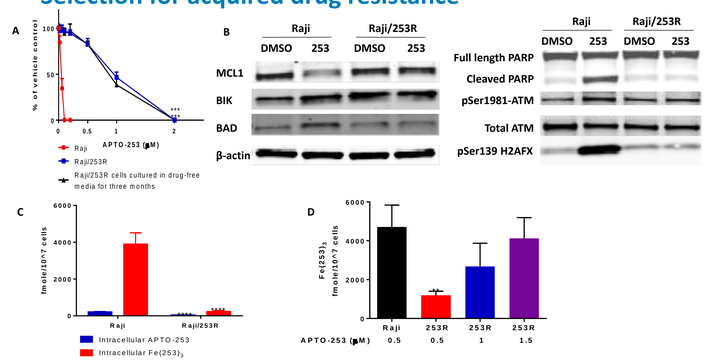
BRCA1 isogenic pair cell lines



BRCA2 isogenic pair cell lines



Selection for acquired drug resistance



(A) Concentration-survival curves for Raji, Raji/253R and Raji/253R cells after culture in drug-free medium for 3 months . (B) Western blot analysis of proteins involved in apoptosis and DNA damage in Raji and Raji/253R treated with DMSO or APTO-253 0.5 μM for 24 h. (C) The intracellular accumulation of APTO-253 and Fe(253)$_3$ in Raji and Raji/253R cells after a 6 h exposure to 0.5 μM APTO-253. (D) The intracellular accumulation of Fe(253)$_3$ in the Raji and Raji/253R cells at 6 h as a function of APTO-253 concentration.

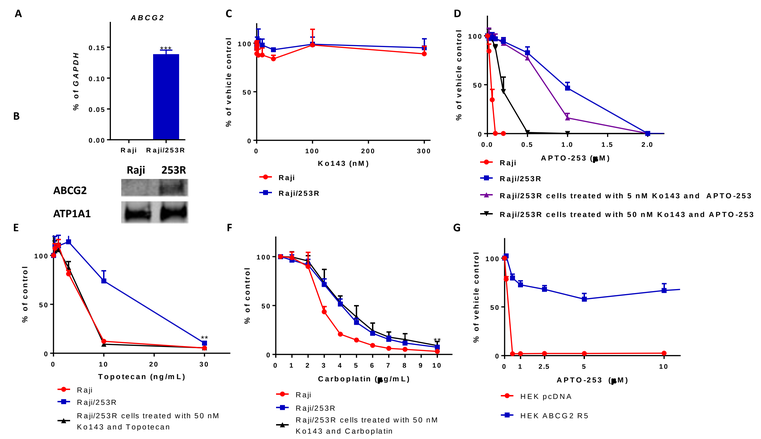
Mechanism of drug resistance

Rank order of genes up-regulated in Raji/253R cells.

Gene name	Fold increase	Adjusted p value
ABCG2	1127.3	2.24E-05
BCHE	173.8	3.24E-05
HUNK	52.9	8.59E-04
RIMBP2	46.6	3.99E-04
AK8	42.5	5.74E-04

ABC transporter family member genes up-regulated in Raji/253R cells.

Gene name	Fold increase	Adjusted p value
ABCG2	1127.3	2.24E-05
ABCG1	0.9	8.56E-01
ABCB1 (MDR1)	4.8	8.24E-04
ABCC1 (MRP1)	1.5	1.25E-02
ABCC2 (MRP2)	3.2	6.09E-03



(A,B) Relative levels of ABCG2 mRNA and protein in the Raji and Raji/253R. (C) Cytotoxicity of Ko143 in Raji and Raji/253R . (D) Concentration-survival curves for Raji and Raji/253R treated with APTO-253 alone or in combination with APTO-253 and 5 nM or 50 nM Ko143. Cytotoxicity of topotecan (E) and carboplatin (F) in Raji and Raji/253R and the combination with 50 nM Ko143 in Raji/253R. (G) Concentration-survival curves for HEK-293 transfected with pcDNA and ABCG2, clone R5 treated with APTO-253.

CONCLUSIONS

- **Treatment of cells with APTO-253 caused DNA damage. Loss of either BRCA1 or BRCA2 function in multiple isogenic paired cell lines resulted in hypersensitivity to APTO-253 of a magnitude similar to the effects of PARP inhibitors, olaparib.**
- **RNA-seq analysis revealed that over-expression of the ABCG2 drug efflux pump is a key mechanism of resistance.**
- **APTO-253 joins the limited repertoire of drugs which can exploit defects in homologous recombination and is of particular interest because it does not produce myelosuppression.**